Genetics and Molecular Biology, 28, 2, 214-217 (2005)

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Short Communication

Study of the CCR5-m303 mutation in three different ethnic groups from Brazil

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Abstract

The main coreceptor gene involved in HIV-1 infection is CCR5 β chemokine receptor gene for which several mutations have been described, some of which have correlated with HIV-1 infection, acquired immune deficiency syndrome (AIDS), or both. Deletion of 32bp in the CCR5 gene (Δ32) has been shown to confer resistance to infection by HIV-1 R5 strains. Another mutation, characterized by a thymine to adenine (T to A) nucleotide substitution at position 303 (m303), has shown the same effects as the Δ32 mutation, with previous studies having shown that the allele frequency of the CCR5-m303 mutation is 0.014 in African-American and 0.007 in French populations. The Brazilian population is known to be genetically diverse, because of which we investigated the allele frequency of the CCR5-m303 mutation in three different Brazilian ethnic groups containing individuals who were not infected with HIV-1 and also in a cohort of HIV-1 long-term non-progressors. We used the polymerase chain reaction (PCR) and *Hinc*II restriction fragment length polymorphisms (RFLP) to investigate these populations and found that none of the 566 individuals examined the mutant CCR5-m303 allele. These results are in accordance with the previously reported allelic frequencies for African-American and Caucasian populations and may reflect the real prevalence of the m303 mutation in Brazil.

Key words: CCR5 gene, m303 frequency, Brazilian populations, HIV-1.

Received: July 7, 2004; Accepted: December 14, 2004.

The β chemokine receptor CCR5, a member of the seven trans-membrane G-protein-coupled receptor family (Berger *et al*.1999), has been identified as a main coreceptor for entry of R5-tropic HIV-1 strains into target cells (*e.g*. $CD4^+$ T- cells, monocytes/macrophages) (Alkhatib *et al*. 1996; Choe *et al*. 1996; Deng *et al*. 1996; Dragic *et al*. 1996; Doranz *et al*. 1996). The majority of HIV-1 variants occurring during the asymptomatic infection period are CCR5 tropic and are more frequently associated with *in vivo* transmission (Zhu *et al*. 1993). In contrast, the CXCR4 α-chemokine receptor is the coreceptor for the X4-tropic variants often found in the late period of infection (Feng *et al*. 1996).

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Several studies have shown that mutations in the CCR5 gene affect both the HIV-1 infection process and the progression to AIDS. A 32 bp deletion (Δ32) in the CCR5 coding region has been shown to confer resistance to infection by R5 strains of HIV-1 (Dean *et al*. 1996; Liu *et al*. 1996). Moreover, as compared with peripheral blood mononuclear cells (PBMCs) from CCR5/CCR5 (wild-type) homozygotes, PBMCs from CCR5/Δ32 heterozygotes are less susceptible to *in vitro* infection by HIV-1 R5 strains (Deng *et al*. 1996; Berger *et al*. 1999; Grimaldi *et al*.2002), with seropositive CCR5/Δ32 individuals having shown delayed progression to AIDS (Dean *et al*. 1996; Huang *et al*. 1996).

Another mutation in the CCR5 gene is the m303 mutation, characterized by an open reading frame single T to A base pair transversion at nucleotide 303 which indicates a cysteine to stop codon change in the first extracellular loop

of the β-chemokine receptor protein at amino acid 101 (C101X) (Carrington *et al*. 1997). The m303 mutation appears to play the same role as the Δ32 deletion while mutagenesis assays have not detected the expression of the m303 coreceptor on the surface of CCR5 null transfected cells which were found to be non-susceptible to HIV-1 R5-isolates in infection assays (Blanpain *et al*. 2000).

The m303 mutation has only been detected in heterozygosis in 2.86% (1/35) of African-American and 5.56% (1/18) of French (who also possessed the Δ32 allele in compound trans-heterozygosis) individuals at high risk for HIV-1 infection (Carrington *et al*. 1997; Quillent *et al*. 1998), indeed, Quillent *et al*. (1998) found as few as 1.4% of blood donors of undefined ethnic origin possessed the m303 mutation

Ometto *et al*. (1999) observed an m303 allelic frequency of 0.002 for uninfected Italian infants born to HIV-1 seropositive mothers and 0 for HIV-infected Italian children. The mutant m303 allele was not identified in 200 Kuwaitis (Voevodin *et al*. 1999), nor among 687 South Africans (Williamson *et al*. 2000) or in seronegative but HIV-1 infected Chinese (Shieh *et al*.1999; Wang *et al*. 2003), although the m303 gene was detected at an allelic frequency of 0.007 in 145 Caucasians (Williamson *et al*. 2000).

Since there is a great deal of genetic diversity in Brazilian human populations but a lack of reports concerning the m303 allele, we investigated the frequency of this allele in three different Brazilian seronegative ethnic groups and in a cohort of HIV-1 long-term non-progressors.

One seronegative group was from the city of Salvador, the capital of the northeastern Brazilian state of Bahia, where 80% of the 2.5 million inhabitants (Anon, 2000) are Afro-Brazilians or of mixed African and Portuguese (mestizo) descent (Azevedo *et al*. 1982). To form this group we randomly selected 400 people from a cross-sectional survey population from sentinel surveillance areas previously established for the investigation of various infectious diseases (Teixeira *et al*. 2002).

Another seronegative group was from the city of Joinville, the capital of the southern Brazilian state of Santa Catarina, the majority of the approximately 430,000 of this city being of German descendent (Anon, 2000). This group was composed of 50 blood-donors of German ancestry who had no reported risk behavior for sexually transmitted diseases.

A further seronegative group consisted of 50 individuals from each of two Amerindian tribes (the Tiriyó and the Waiampi) located in the northern Amazon river basin. The Tiriyó tribe speaks the Caribe language and inhabits a reservation on the Suriname-Brazil frontier, 750 of its 1,700 members living in Brazil, while the Waiampi tribe speaks Tupi-Guarani and lives on a reservation on the French Guiana-Brazil frontier with 450 of its 1,200 members living in the Brazilian state of Amapá. All the Amerindians (total = 100) included in the survey were HIV-1 negative.

The seropositive group consisted of 16 HIV-1 infected long-term non-progressor (LTNP) individuals selected from an outpatient clinic at the Federal University Hospital in Salvador, the inclusion criteria being 10 years or more of asymptomatic infection with no use of anti-retroviral therapy and CD4 counts above 500 cells/mm^3.

Blood samples, collected with the informed consent of the participants, were obtained from the seronegative Amerindian groups between April and May 1997 and from the seronegative Salvador and seropositive HIV-1 groups between April and August 1998, the Joinville seronegative group being sampled between November 2000 and January 2001. Each individual provided 10 ml of blood, which was collected as described by Grimaldi *et al*. (2002) using ethylene-diamine tetra acetic acid (EDTA) as an anticoagulant. The blood plasma from each of the participants was screened for HIV types 1 and 2 using an enzyme-linked immunosorbent assay (ELISA) (Enzygnost® Anti HIV-1/2 Plus-Behring, Marburg, Germany). Repeatedly reactive samples were submitted to Western Blotting (HIV Blot 2.2, Genelab Diagnostics, Singapore Science Park, Singapore) and were interpreted according to the manufacturer's instructions.

We extracted DNA from PBMCs and whole blood using a commercial kit (DNAzol, GIBCO-BRL, Rockville, USA). The CCR5-m303 gene was amplified by the PCR method described by Carrington *et al*. (1997) using 100 ng of sample DNA and a Perkin-Elmer 9600 thermal cycler (Perkin-Elmer, Connecticut, USA). The m303 mutation generates a premature stop codon in the CCR5 gene which deactivates the *Hinc*II restriction site, which means that the *Hinc*II restriction enzyme can be used to detect the m303 mutation. We used the *Hin*cll 5U restriction enzyme (BioLabs Inc., New England, USA.) to digest the PCR products in a final volume of 10 μL and an incubation period of three hours at 37 °C. The RFLP products were separated by electrophoresis on 1.5% agarose gel and visualized by standard techniques. The CCR5/CCR5 wild-type genotype was detected by 240 and 46 bp bands while CCR5/m303 was represented by 286, 240 and 46 bp; and m303/m303 by a single band of 286 bp.

We did not detect the m303 allele in any of the 566 individuals sampled, showing that his mutation was absent from the four groups studied (Figure 1).

It has been suggested (Winkler *et al.*, 2004) that infectious diseases can exert a selective advantage on some mutations, and it may be that the m303 allele is currently being subjected to such a process and spreading through populations of different ethnic origin as a result of its influence on HIV-1 infection and progression to AIDS. In our study there was no evidence of the presence of the m303 allele in any of the groups, including the HIV-1 LTNP group,

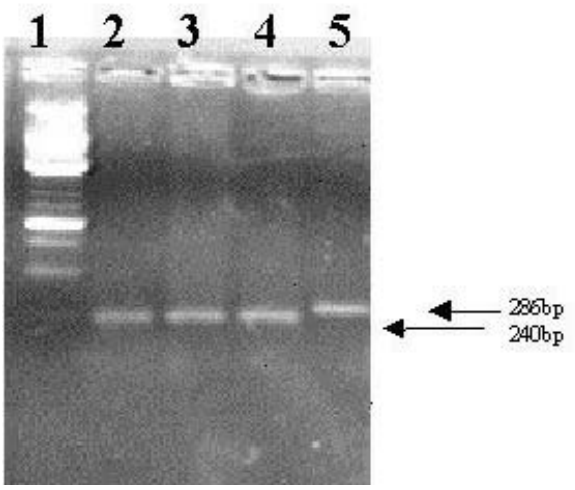

Figure 1 - Agarose gel electrophoresis of RFLP *Hinc*II digested PCR amplified DNA. Lane 1 = 250 bp molecular weight marker; Lanes 2, 3 and 4 = 240 bp digested fragment from wild-type homozygotes (CCR5/CCR5); Lane 5 = 286 bp non-digested fragment (negative control).

suggesting that this event has not reached detectable levels yet.

Our results for the Salvador seronegative group (80% of African-Brazilian or mestizo origin are in agreement with those published by Carrington *et al*. (1997) for a cohort of highly exposed uninfected individuals and by Williamson *et al*. (2000) who found no evidence of the m303 allele in individuals of African origin. Although previous reports have detected the m303 allele in Caucasians (Quillent *et al*. 1998; Williamson *et al*. 2000) we did not detect it in the Joinville seronegative group, which consisted of blood donors of German descent. This could be because the sample-size (50 individuals) was small or because of miscegenation. Since previous studies (Voevodin *et al*. 1999; Shieh *et al*.1999; Wang *et al*. 2003) have not detected the m303 allele in Asian populations we did not expect to find it in either the Waiampi or Tiriyó populations because they are of Asian descent, and this was indeed the case.

In summary, our data indicates that the m303 allele is not prevalent in three HIV-1 seronegative Brazilian populations of different ethnic origin nor does it occur in a group of long-term non-progressors from Salvador.

Acknowledgement

This work was partially supported by the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), and by Coordenação de Aperfeiçoamento de Pessoal em Nível Superior (CAPES).

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Associate Editor: Francisco Mauro Salzano